ELSEVIER

Gene Expression Patterns 8 (2007) 1–11

G≡P

www.elsevier.com/locate/gep

Gene expression of the repulsive guidance molecules/neogenin in the developing and mature mouse visual system: C57BL/6J vs. the glaucoma model DBA/2J

Sven Schnichels [a,b,1,2], Sabine Conrad [c,1], Katrin Warstat [d], Sigrid Henke-Fahle [b], Thomas Skutella [c], Ulrich Schraermeyer [a], Sylvie Julien [a,b,*,3]

[a] *Section for Experimental Vitreoretinal Surgery, University Eye Hospital, Schleichstr. 12/1, 72076 Tübingen, Germany*
[b] *University Eye Hospital, Schleichstr. 12/1, 72076 Tübingen, Germany*
[c] *Institute of Anatomy, Department of Experimental Embryology, Tissue Engineering Division, Österbergstr. 3, 72074 Tübingen, Germany*
[d] *Center for Orthopaedic Surgery, University Hospital Tübingen, Research Laboratory, Waldhörnlestr. 22, 72076 Tübingen, Germany*

Received 28 February 2007; received in revised form 6 July 2007; accepted 9 September 2007
Available online 18 September 2007

Abstract

We used *in-situ* hybridization to analyze the expression patterns of three known members (a, b and c) of the RGM (“repulsive guidance molecule”) gene family and of the RGMa receptor neogenin in a glaucoma mouse model (DBA/2J strain) and the C57BL/6J strain, which served as a control. In order to understand the role of the RGMs and neogenin in glaucoma, we characterized their expression patterns in the developing and mature mouse retina and in the optic nerve. In all investigated stages from post-natal day (P) 0 to 15 months (M) RGMa, RGMb and neogenin expression was detected in the ganglion cell layer (GCL). From P10 to 15 M, we found RGMa, RGMb and neogenin expression in the inner nuclear layer (INL) and the outer nuclear layer (ONL). In P10- and older mice, the expression patterns of RGMa and its receptor neogenin were similar, while that of RGMb differed from both. As expected, no specific retinal expression of RGMc was detected in any of the age groups investigated. C57BL/6J mice and DBA/2J mice displayed no differences in the expression pattern of RGMa, RGMb, RGMc and neogenin in the developing retina (gestational age 14.5 days (E14.5), P0 & P10). Interestingly, we found a higher expression of RGMa, RGMb and neogenin in the retinas of all glaucoma-affected mice than in the age-matched control strain. Furthermore, we detected a higher RGMa and RGMb expression in the optic nerves of glaucoma-affected DBA/2J-mice older than 11 M than in C57BL/6J mice of the same age.

Keywords: Repulsive guidance molecules; Neogenin; Visual system; Glaucoma mouse model; *In-situ* hybridization

1. Results and discussion

The repulsive guidance molecule (RGM), a novel axon guidance protein of 33 kDa, has been cloned and functionally characterized in the chick (RGMa) as a molecular determinant for retinotectal map formation (Monnier et al., 2002). It has been demonstrated that recombinant RGMa induces collapse of temporal but not nasal growth cones of retinal ganglion cells (RGCs) and guides temporal retinal axons *in vitro*, demonstrating a repulsive and axon-specific guiding activity (Monnier et al., 2002). The molec-

* Corresponding author. Address: Section for Experimental Vitreoretinal Surgery, University Eye Hospital, Schleichstr. 12/1, 72076 Tübingen, Germany. Tel.: +49 7071 29 84888; fax: +49 7071 29 4554.
E-mail address: Sylvie.Julien@med.uni-tuebingen.de (S. Julien).
[1] These authors contributed equally to this work.
[2] Sven Schnichels was supported by the Else Kröner-Fresenius foundation.
[3] Sylvie Julien was supported by the fortüne program of the University Hospital of Tübingen.

1567-133X/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.modgep.2007.09.002

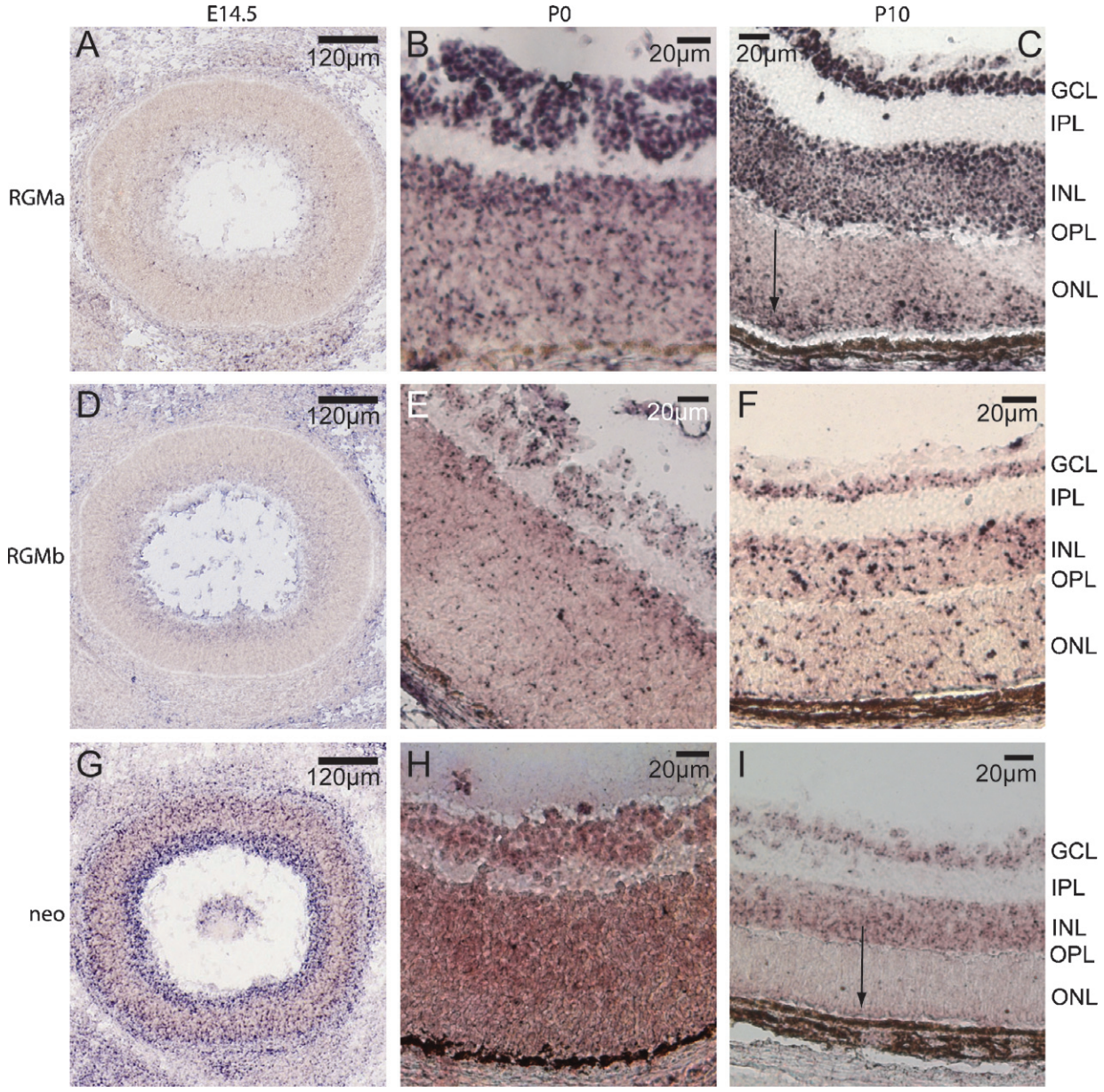

Fig. 1. RGMa expression in the developing mouse retina: no RGMa RNA was detected in the embryonic mouse eye (A). RGMa was expressed in every layer of the developing retina in P0 mice except for the inner plexiform layer (IPL) (B). The typical RGMa expression pattern with signals in the ganglion cell layer (GCL), the inner nuclear layer (INL) and the outer nuclear layer (ONL) was visible from post-natal day 10 (P10) on (C). Higher expression localized near the outer border of the ONL (probably cones) is indicated by an arrow. RGMb expression in the developing mouse retina: RGMb showed a very weak expression in the inner tissue of the embryonic retina (D). RGMb RNA was detected in every layer of the developing retina in P0 mice except for the IPL (E). P10 mice showed the typical spotty RGMb expression pattern of mature mice, with expression in the GCL, the INL and the ONL (F). Neogenin expression in the developing mouse retina: neogenin RNA was detected in a gradient decreasing from the inside to the outside in the E14.5 mouse embryo (G). Neogenin was expressed in every layer in the P0 mouse except for the IPL (H). Neogenin RNA was identified in the GCL, the INL and, to a lesser extent, in the ONL. As with RGMa, a higher neogenin expression within the ONL was detected in the tissue close to the outer limiting membrane (arrow) (I).

ular structure of RGMs includes an N-terminal signal sequence, an RGD motif, a partial von Willebrand factor type D domain, and a C-terminal GPI-anchor domain. Recently, RGMb was identified in the chick genome (GeneID: 427277). In contrast, three genes have been isolated in the mouse genome to date (Schmidtmer and Engelkamp, 2004). None of the three murine members of this protein family share significant sequence homology with any other known guidance molecules. The expression patterns of the three homologues were identified in the mouse embryo by *in-situ* hybridization (Oldekamp et al., 2004; Schmidtmer and Engelkamp, 2004). RGMa and RGMb are predominantly expressed in the developing and adult central nervous system (CNS) in complementary patterns with little overlap. In the retina, RGMb but not RGMa or RGMc is expressed in the RGCs (Niederkofler et al., 2004). RGMc

expression is restricted to all striated muscles and the myocardium.

In order to address, which of the mouse RGM family members might play a dominant role in the development of the retinocollicular system, Niederkofler and colleagues (2004) performed *in-situ* hybridization experiments in the mouse superior colliculus at post-natal day (P) 0. This is the stage just before targeting of RGC axons to defined anteroposterior positions occurs (Simon and O'Leary, 1992). They found that RGMa was prominently expressed in the superior colliculus at this stage. Recently, neogenin, which was known to be a netrin-1 receptor, was also demonstrated to function as an RGMa receptor (Rajagopalan et al., 2004). A study in chick embryos revealed that RGMa and its receptor neogenin regulate neuronal cell survival (Matsunaga et al., 2004). Thus, neogenin is a dependence receptor inducing cell death in the absence of RGMa, whereas the presence of RGMa inhibits this effect.

Evidence is now emerging that the proteins governing developmental axon guidance may contribute to the failure of injured central neurons to regenerate (Wizenmann et al., 1993). The expression of chemorepulsive semaphorins and ephrins has been shown to correlate with the success or failure of injured axons to regenerate (Pasterkamp and Verhaagen, 2001; Knoll and Drescher, 2002) in the damaged CNS. As a step toward elucidating the role of chemorepul-

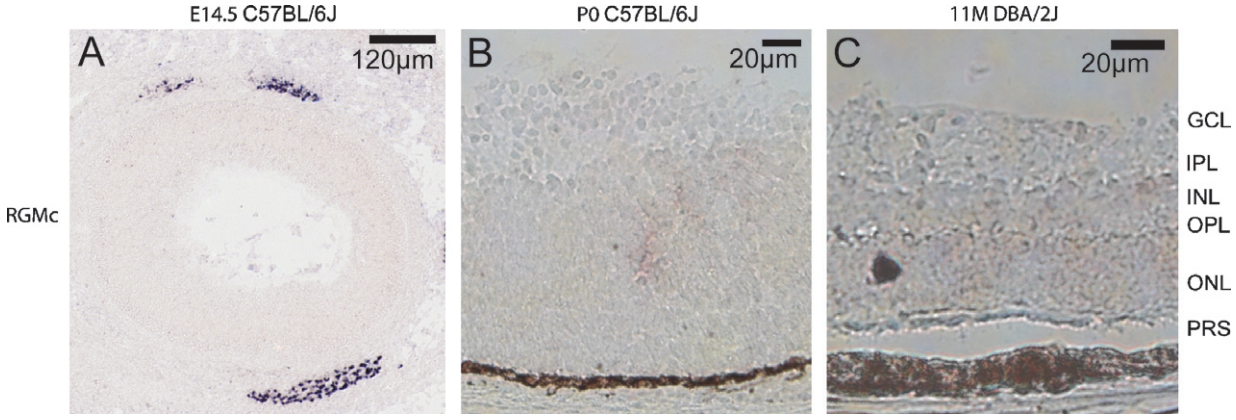

Fig. 2. Absence of RGMc expression in the retina. In all investigated ages, no specific signal was found in the retina. No differences between the two strains were visible (A–C). As expected, a specific signal was visible in the eye muscles (A).

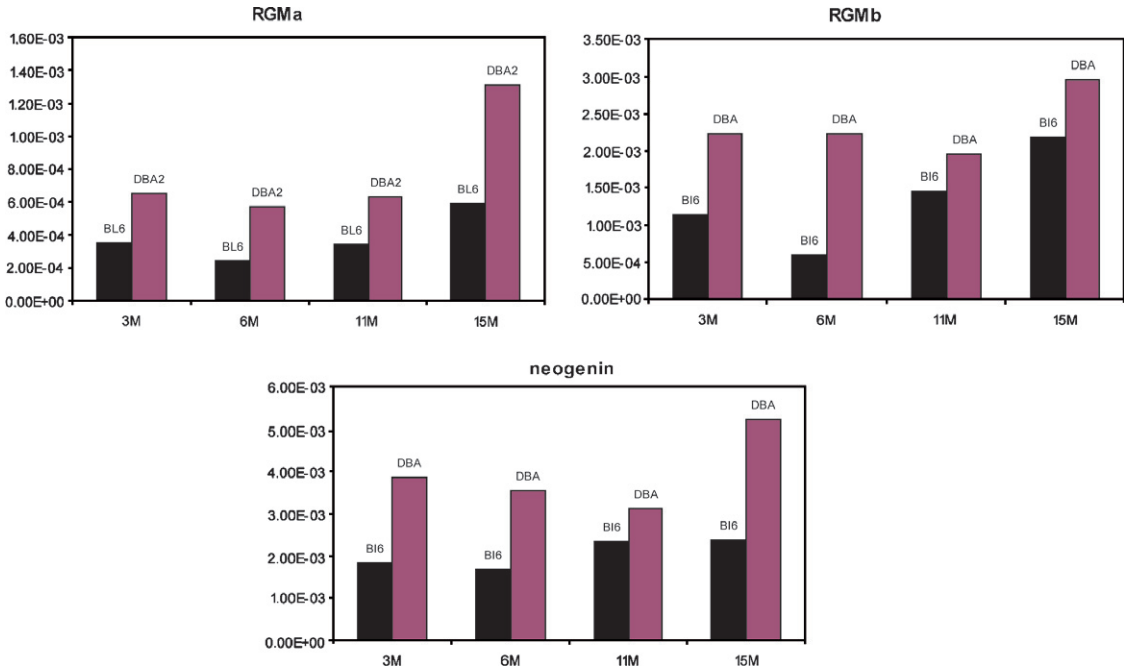

Fig. 3. Quantification of expression of RGMa, RGMb and neogenin in the mature eye and optic nerve. Quantification of RNA expression of RGMa, RGMb and neogenin in samples obtained from whole eyes including the optic nerves. A higher expression of all three genes in DBA/2J-mice (DBA2) compared to the age-matched C57BL/6J (BL6) was obvious throughout all investigated ages.

sive molecules in axonal regeneration, we studied the effect of spinal cord injury on the expression of RGMa in an earlier publication (Schwab et al., 2005). The presence of RGMa in a glial barrier and its inhibitory activity *in vitro* suggest that it might exert inhibitory effects on regenerating axons in the glial scar.

Our aim is now to study the function of RGMs in a genetically determined murine pigmentary glaucoma strain, the DBA/2J mouse (John et al., 1998), in order to provide a basis for the development of strategies to support regeneration processes. Glaucoma results in a slowly progressing decrease in the population of RGCs, the retinal neurons that project to the brain *via* the optic nerve. The DBA/2J mouse provides a powerful model system for determining mechanisms that insult RGCs in glaucoma and for characterizing specific damaging pathways. Indeed, the RGC loss in DBA/2J inbred mice is natural, age-related, variable, with an asynchronous progressive course

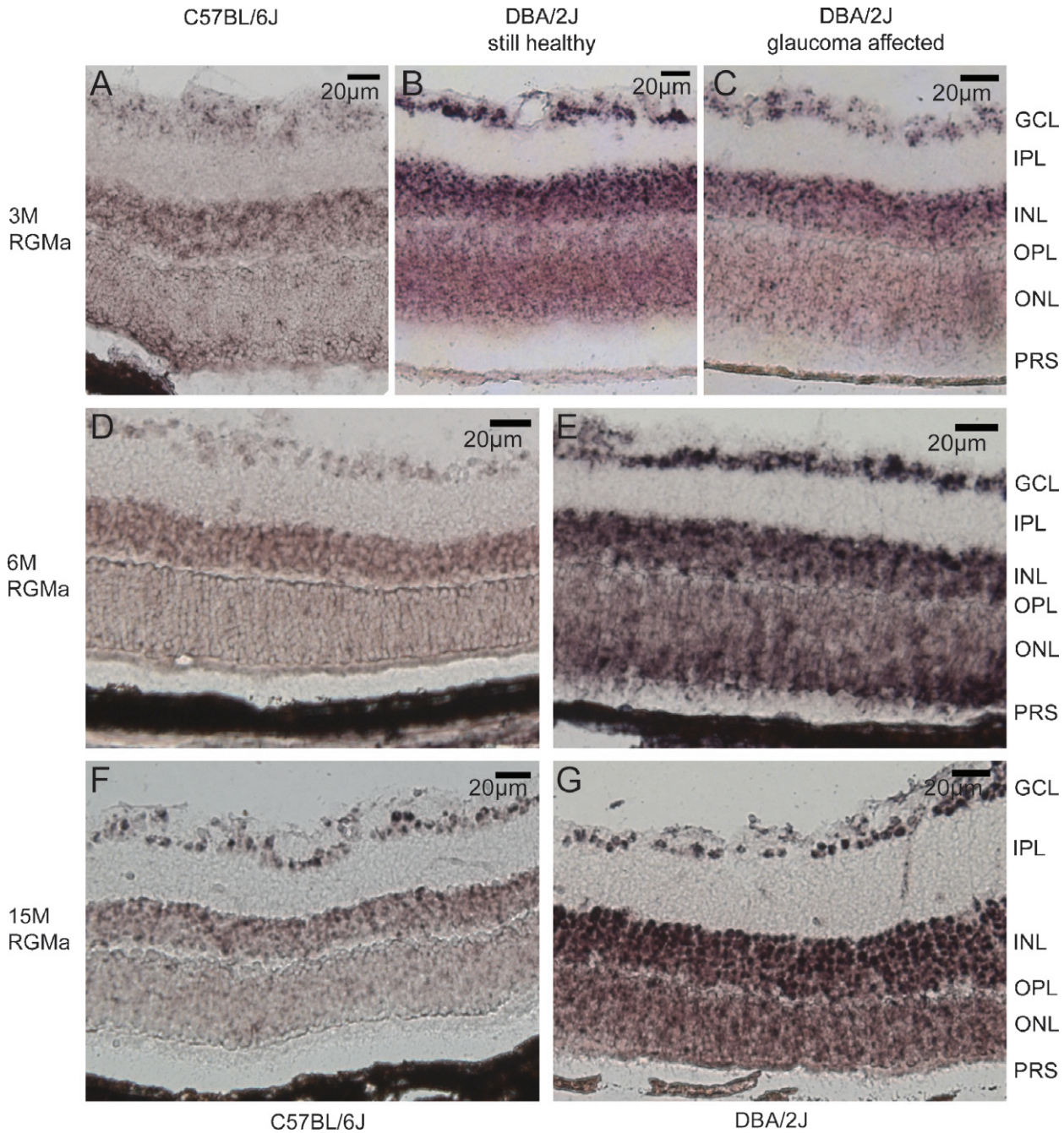

Fig. 4. Differences in the expression of RGMa in mature C57BL/6J and DBA/2J retinas. RGMa expression was found in the GCL, the INL and the ONL in 3-month-old mice of the investigated strains C57BL/6J (A) and DBA/2J (B and C). Expression in the DBA/2J mice was stronger nevertheless an obvious difference existed between the expression intensity of DBA/2J mice not yet affected by glaucoma (B) and those that already were (C). RGMa expression in retinas of 6-month-old and 15-month-old mice is generaly weaker in C57BL/6J mice (D and F) than in DBA/2J mice (E and G).

that in this way resembles the human glaucoma (Libby et al., 2005). The progression of the disease is asymmetric (Schlamp et al., 2006). Both eyes can therefore be treated as independent (Libby et al., 2005). A detailed knowledge of the expression pattern of RGMs and neogenin is an important prerequisite for the development of agents that support regeneration by interfering with or utilizing the effects of these chemotropic molecules. Sites of RGMa, RGMb and RGMc expression have already been analyzed by means of whole mount *in-situ* hybridization at early stages of mouse development and on sectioned brain structures at later stages (Oldekamp et al., 2004; Schmidtmer and Engelkamp, 2004) but have not yet been mapped in sufficient detail in the visual system. In this study, the expression patterns of RGMa, RGMb, RGMc and neogenin were analyzed in the developing and mature visual system of control C57BL/6J mice and DBA/2J mice by *in-situ* hybridization. The expression patterns of these transcripts were analyzed in the developing visual system from the time point when axons entered the optic nerve to the first post-natal weeks and to determine whether RGMs are correlated with the advancement of the RGC death process and the increase of intraocular pressure in DBA/2J mice at 3, 6, 9, 11 and 15 M of age.

1.1. RGMa, RGMb and neogenin expression in the developing mouse retina

There were no differences in the expression pattern of RGMa, RGMb, RGMc and neogenin in the developing

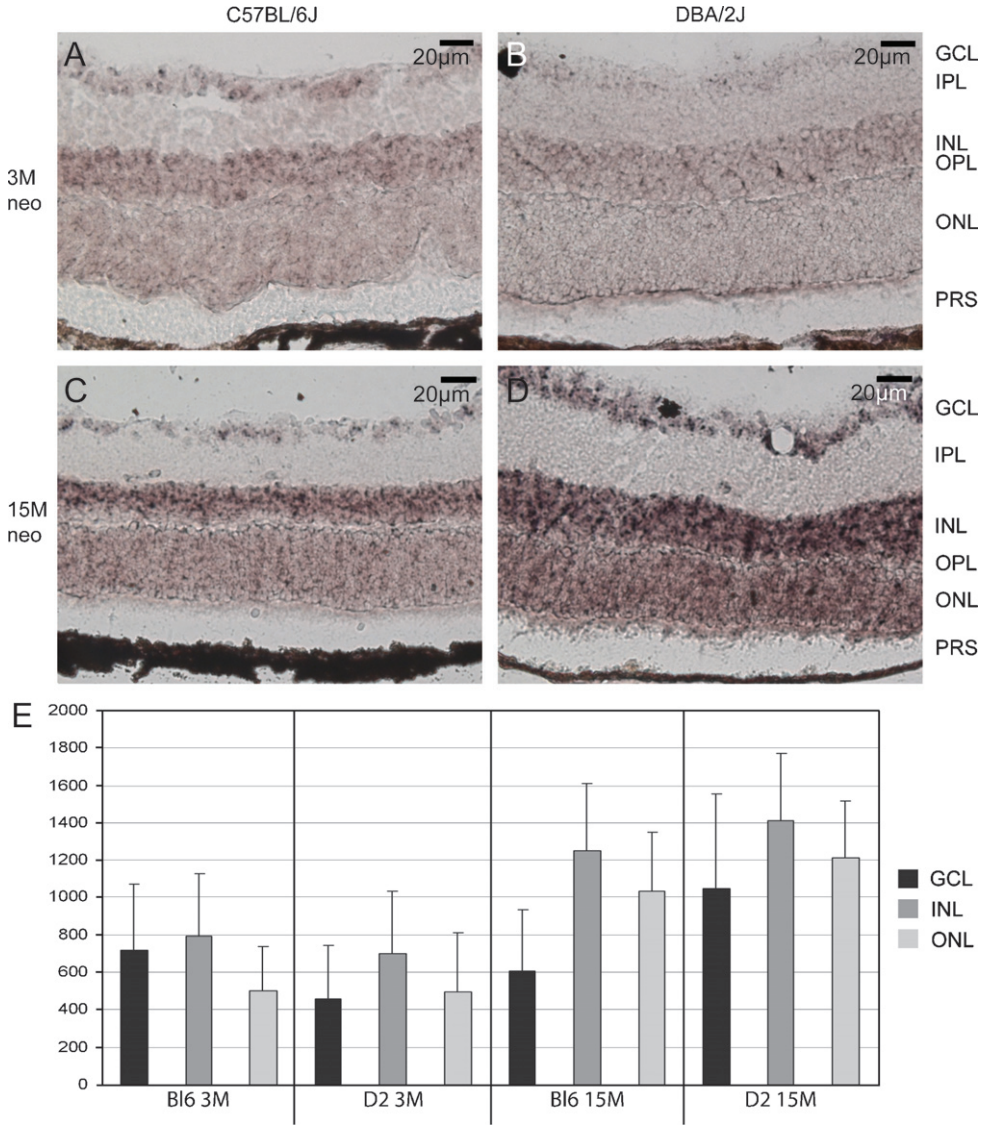

Fig. 5. Differences in neogenin expression in the retinas of aging C57BL/6J and DBA/2J mice. Neogenin expression in the retinas of 3-month-old C57BL/6J mice (A) was stronger than in DBA/2J mice not yet affected by glaucoma (B). In mice aged 15 M, the strength of expression appeared inverted between the two strains (C vs. D). Quantitative analysis of the sections. The histogram reveals a lower expression in 3-month-old DBA/2J mice (D2 3M) compared to 3-month-old C57BL/6J mice (Bl6 3M). This effect inversed in 15-month-old mice, although the expression rose in both strains (E).

retina between C57BL/6J and DBA/2J mice (E14.5, P0 and P10) (data not shown). Therefore, we do not distinguish between the two strains in the developing retina.

1.1.1. RGMa

In the E14.5 embryo, no RGMa expression was detectable in the tissue destined to be the future retina (Fig. 1A). In P0 mice, expression had spread throughout the whole retina except for the inner plexiform layer (IPL). The strongest RGMa expression was observed in the ganglion cell layer (GCL). A gradient was visible from the inner to the outer retina (Fig. 1B). A strong expression of RGMa was observed in the GCL as well as in the INL in P10 mice, whereas expression in the outer nuclear layer (ONL) was weaker, and a gradient decreasing from the inside to the outside was evident. In addition, RGMa RNA was detected on the outer border of the ONL, probably near the nuclei of the cones (Fig. 1C).

1.1.2. RGMb

RGMb showed only very weak expression in the inner tissue of the future retina of an E14.5 embryo (Fig. 1D). In P0 mice, RGMb was expressed in the same layers as RGMa, but without an obvious gradient of intensity (Fig. 1E). Expression of RGMb in P10 mice differed from that of RGMa. RGMb RNA was detected in the GCL, INL and ONL, but the expression in the INL and the weaker expression in the ONL appeared to be more cell-type specific than RGMa expression. The expression pattern was spotty (Fig. 1F).

1.1.3. Neogenin

Neogenin was present in the whole embryonic retina and exhibited the strongest expression in the embryonic retina compared to RGMa and RGMb. A gradient decreasing from the inner to the outer future retina was also noticeable (Fig. 1G). In P0 mice, neogenin displayed the same expres-

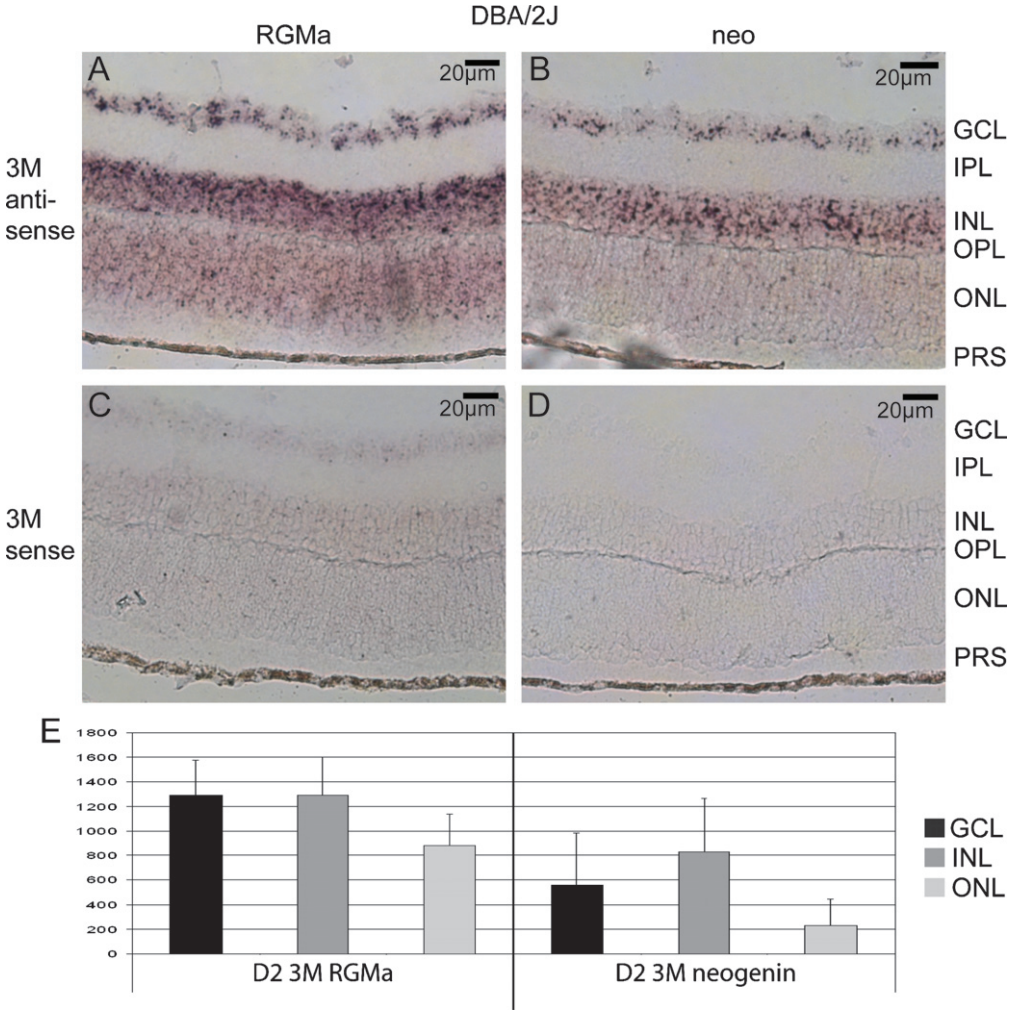

Fig. 6. Congruent expression pattern of RGMa and neogenin in the mouse retina. All investigated ages in both mice strains showed a congruent expression pattern of RGMa (A) and neogenin (B), with a weaker expression of neogenin. This is shown here in retinas of 3-month-old DBA/2J mice. RGMa (C) and neogenin (D) sense probes showed no specific signal. Quantitative analysis of the RGMa-/neogenin expression in retinas of 3-month-old DBA/2J mice. The RGMa-signal in the retina is always stronger than the neogenin-signal (E).

sion pattern as RGMa but without a visible gradient (Fig. 1H). The neogenin expression pattern of P10 mice was congruent with the RGMa expression pattern, but signal intensity was lower. Similar to RGMa, a large amount of neogenin RNA was evident between the ONL and the developing photoreceptor segments (PRS), i.e. in the vicinity of the outer limiting membrane (Fig. 1I).

1.1.4. RGMc

No specific retinal expression of RGMc was detected at any age investigated (Fig. 2).

1.2. Phenotypic differences between healthy (C57BL/6J) and glaucoma mice (DBA/2J)

Our investigation showed that in mice aged between 3 and 9 M differences in expression between the DBA/2J strain and C57BL/6J mice depend less on age than on the onset of the disease. In older mice between 11 and 15 M, the expression pattern was always very similar due to the fact that all the mice suffered from glaucoma by this age. No difference expression pattern was found in C57BL/6J and DBA/2J mice, however, expression intensity in DBA/2J mice was generally stronger, depending on the advancement of the disease.

1.2.1. Expression of RGMa, RGMb and neogenin in the complete eye including the optic nerve

To confirm the expression of RGMa, RGMb and neogenin in the mature eye and optic nerve and to determine whether a difference exists in the expression of the three genes between the C57BL/6J and DBA/2J strains, we performed RT-PCRs. Therefore, we investigated the complete eyes including the optic nerves from 3-, 6-, 11- and 15-month-old mice from both strains. In all investigated ages, we found a higher expression of RGMa, RGMb and neogenin in DBA/2J mice compared to C57BL/6J mice (Fig. 3). These results have now to be specified separately for each layer of the retina and the optic nerve.

1.2.2. RGMa, RGMb and neogenin expression in the mature mouse retina

1.2.2.1. RGMa. In the adult animal, RGMa expression was restricted to the GCL and the nuclear layers, whereas the optic fiber layer and the plexiform layers were devoid of label, except for the area near the outer border of the ONL in some mice. In every stage investigated, RGMa expression was weaker in the ONL than in the GCL or INL (Fig. 4A). We found an obvious difference between DBA/2J mice which are still healthy (Fig. 4B) and DBA/2J mice already suffering from glaucoma (Fig. 4C). The expression appeared to be weaker in the glaucoma-affected DBA/2J mice than in the DBA/2J mice, which did not yet have glaucoma. In 3-month-old mice, the level of expression in glaucoma affected DBA/2J mice was 2.3 times higher in the GCL and between 1.1 and 1.3 times higher in the ONL and INL than in the C57BL/6J mice, respectively. With increasing age, the expression of RGMa in C57BL/6J decreased in both the INL and the ONL (Fig. 4A, D and F), in contrast to DBA/2J mice, where the expression

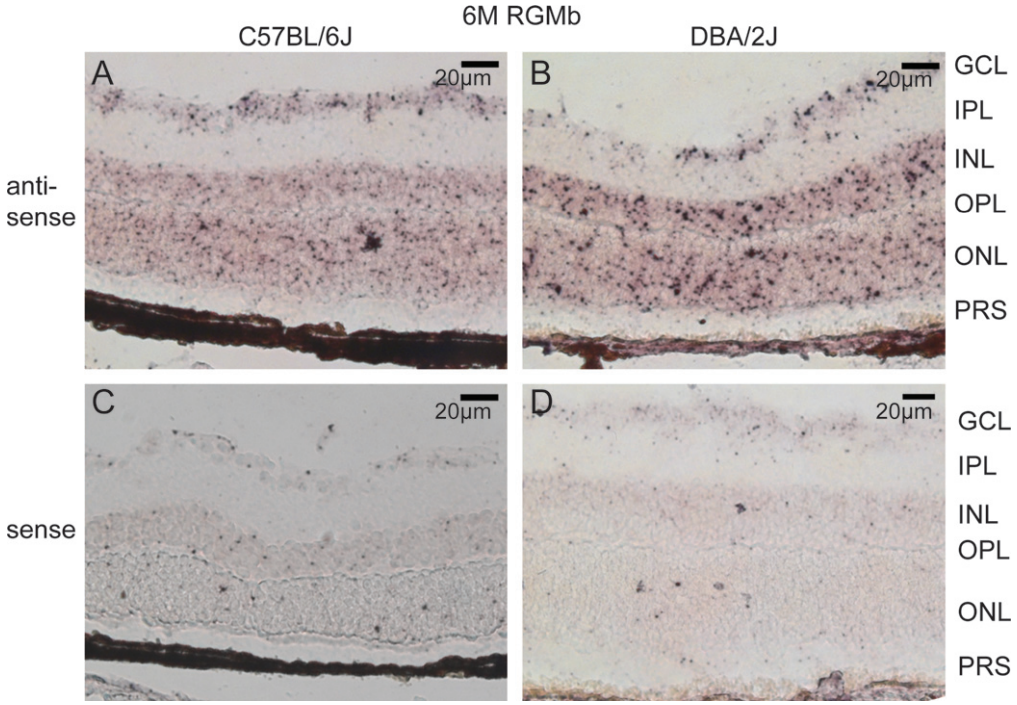

Fig. 7. Differences in RGMb expression in the retinas of C57BL/6J and DBA/2J mice. Example of the typical RGMb expression pattern detected in 6-month-old C57BL/6J (A) and DBA/2J (B) mice. RGMb expression is always higher in the DBA/2J strain. In 6-month-old DBA/2J mice, 25% more RGMb positive cells were found in the ganglion cell layer, the inner and the outer nuclear layer as compared to the retinas of C57BL/6J mice. Sense probes of RGMb showed no specific signal neither in the C57BL/6J (C) nor in the in DBA/2J mice (D).

did not decrease at all with age (Fig. 4B, C, E and G). We observed the highest RGMa expression in the retina sections of 6-month-old DBA/2J mice, an age when glaucoma begins in most of the mice. Quantification of the signal intensity revealed a 3.0 times higher expression in the GCL, a 1.9 higher expression in the INL and a 2.4 higher expression in the ONL of 6-month-old mice, respectively. In 15-month-old mice, the difference was not so striking. We observed a 1.9 times higher expression in the GCL and ONL, and a 1.4-fold higher expression in the INL

1.2.2.2. Neogenin. The intensity of neogenin expression in C57BL/6J mice remained at approximately the same level from 3 to 15 M of age with a slight increase in the INL (Fig. 5A, C and E), whereas in DBA/2J mice the expression of neogenin rose with advancing age/progress of the disease (Fig. 5B, D and E).

1.2.2.3. Comparison between RGMa and neogenin. The expression pattern of RGMa was congruent with the expression pattern of its receptor neogenin, but the intensity of expression was weaker in all investigated ages and in all three layers where signals were found (Fig. 6A, B and E).

1.2.2.4. RGMb. As described for RGMa and neogenin, RGMb displayed the same increase in expression with advancing glaucoma (data not shown). However, as shown for the expression pattern of RGMb in P10 mice, this pattern differed in all investigated ages from the RGMa/neogenin expression pattern. RGMb seemed to be expressed only by a subpopulation of retinal cells (Fig. 7A and B). Further tests with antibodies against RGMb and specific markers against the different retina cell types are necessary to identify the subpopulation.

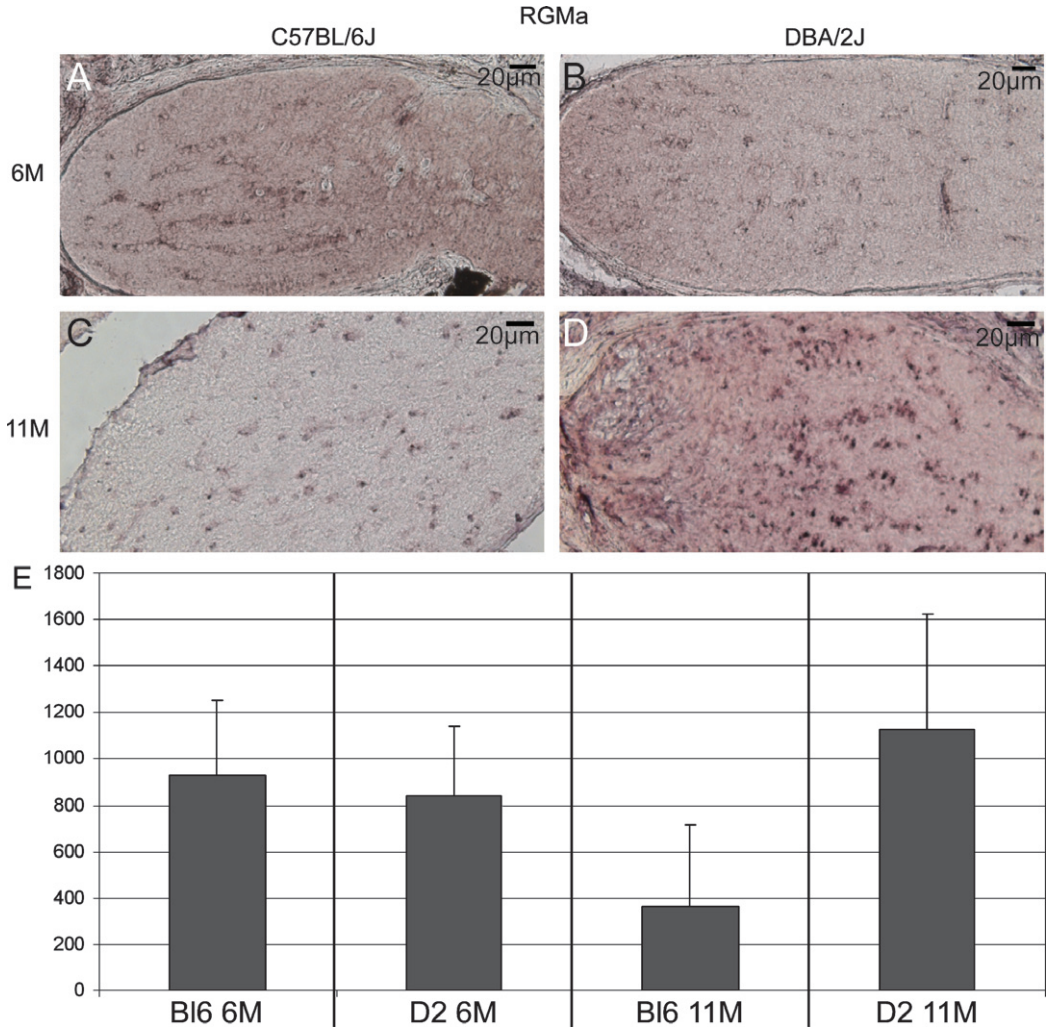

Fig. 8. Differences in RGMa expression in the optic nerves of aging C57BL/6J and DBA/2J mice. No significant difference in the expression of RGMa was found in the optic nerves of 6-month-old C57BL/6J mice (A) and DBA/2J mice (B). C57BL/6J mice older than 11 M (C) showed a lower expression than mice younger than 11 M, whereas the expression increased in DBA/2J mice aged 11 M (D). Quantitative analysis of the optic nerve sections (E). The RGMa signal was equal in 6-month-old mice, whereas the expression in 11-month-old mice was almost 3 times higher in DBA/2J (D2) mice.

1.2.2.5. RGMc. No expression of RGMc was found in any of the investigated age groups (Fig. 3).

1.3. RGMa, RGMb and neogenin expression in the optic nerve

1.3.1. RGMa

Below the age of 10 M, C57BL/6J mice showed a slightly stronger expression of RGMa than DBA/2J mice (Fig. 8A, B and E). The signal intensity in aging C57BL/6J mice decreased (Fig. 8A vs. C; E) in contrast to DBA/2J mice, in which the expression rose (Fig. 8B vs. D; E). The later onset of a higher expression in the optic nerve compared to the retina correlates with apoptosis of neurons after 10 M in the glaucoma model.

1.3.2. RGMb

No differences in the expression pattern of RGMb were found between DBA/2J mice and C57BL/6J mice aged 6 M (Fig. 9A, B and E). However, in older DBA/2J mice (11 M and beyond) expression was stronger than in C57BL/6J mice aged 11 M or older (Fig. 9C–E). Expression of RGMb decreased in aging C57BL/6J mice (Fig. 9A, C and E), whereas expression in DBA/2J mice became very prominent with age (Fig. 9B, D and E).

1.3.3. Neogenin

In mice younger than 10 M, the expression of neogenin in the optic nerve was slightly higher in C57BL/6J mice than in DBA/2J mice. The expression in 11 M-old mice did not differ significantly between the C57BL/6J and

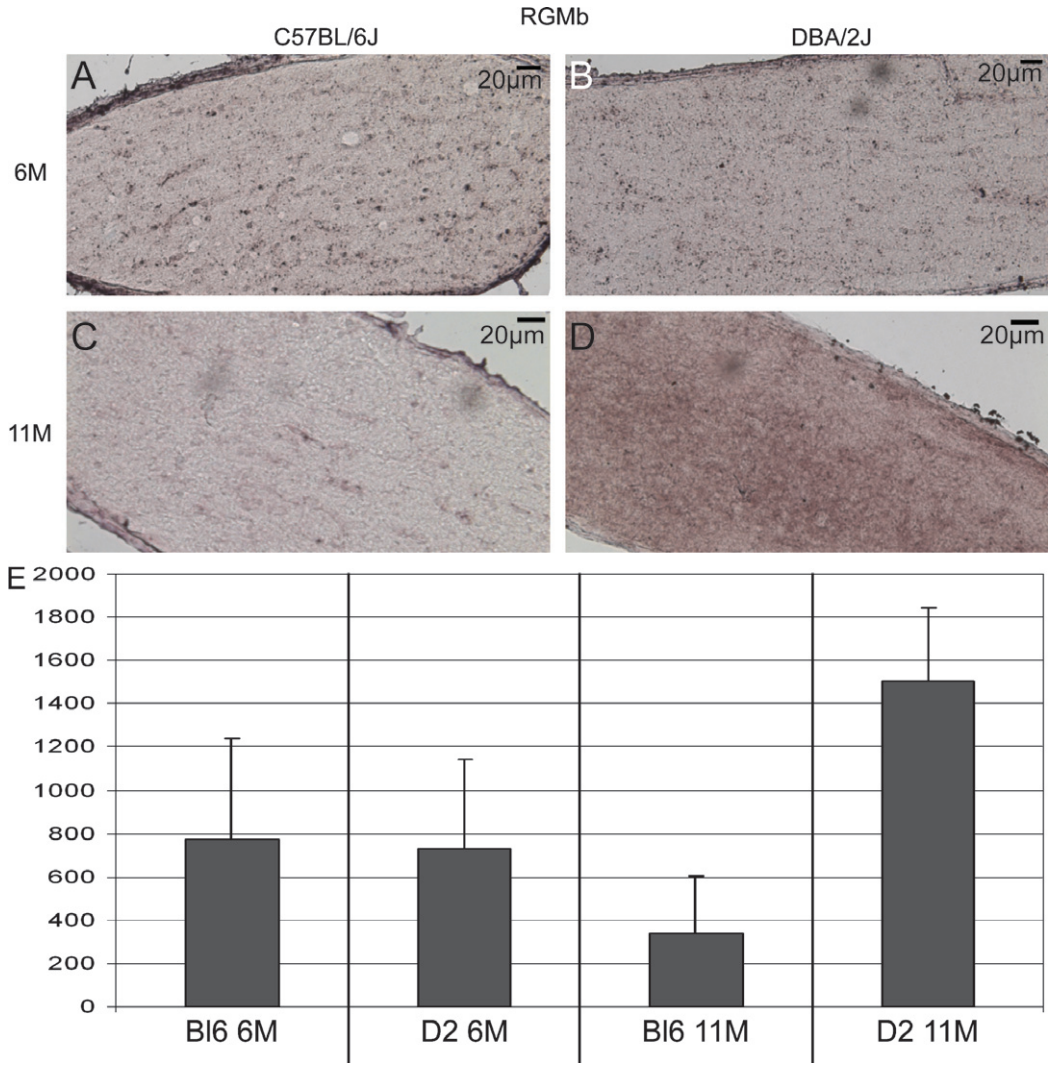

Fig. 9. Differences in RGMb expression in the optic nerves of aging C57BL/6J and DBA/2J mice. No difference in expression intensity was detected between the optic nerves of 6-month-old C57BL/6J mice (A) and those of age-matched DBA/2J mice (B). In C57BL/6J mice older than 11 M (C), expression was lower than in mice younger than 11 M. The expression increased in DBA/2J mice aged 11 M (D) and is highly increased compared to C57BL/6J mice of the same age (D vs. C). The quantitative analysis of the RGMb signal revealed an almost even expression intensity in 6-month-old mice of both strains, whereas the signal intensity in 11-month-old mice was about 5 times higher in DBA/2J (D2) mice (E).

DBA/2J strains. In contrast to RGMa and RGMb, expression of neogenin in the two strains did not invert with age (data not shown).

2. Experimental procedures

2.1. Probes

For RGMa, the fragment HincII (1158)–PvuII 1783) of the Riken clone C230063O06 was cloned into a plasmid and used as a probe. For the RGMb image clone 1139337 was linearized with EcoRI and transcribed with T3 RNA polymerase. For the RGMc, image clone 3497841 was cut with SmaI and transcribed with T7 RNA polymerase. *In-situ* hybridization for neogenin was performed with a 550 bp probe generated by PCR from the 3′ region of GeneID Clone: 81735AI327052.

2.2. Tissue samples

Mouse brains, eyes and optic nerves were prepared and washed in PBS, then immediately frozen in Tissue-Tek O.C.T. in liquid nitrogen. Fourteen-micrometer thick sections were cut on a cryostat (Leica CM 1900) and dried at room temperature.

2.3. In-situ hybridization

The sections were fixed in 4% formaldehyde in PBS for 20 min. Hybridization was performed with dioxigenin-labeled RNA probes. A sense probe served as a control. The *in-situ* hybridization procedure was performed as described previously (Oldekamp et al., 2004). Sections were photographed on an inverted Zeiss microscope (Axiovert 135, Zeiss, Jena, Germany). In order to facilitate comparison between the sections, the microscope settings were not changed between the sections of different animals. In total, 2436 sections from 81 eyes were examined. For RGMa, RGMb and neogenin, between 27 and 38 eyes (105–308 sections) were analyzed for each strain and each anti-sense/sense probe. For RGMc, a total of 40 eyes (142 sections) were examined (see Supplemented data).

2.4. Mice

All mice were handled according to German animal protection laws. All experiments were conducted in accordance with the Association for Research in Vision and Ophthalmology's statement on the use of animals in ophthalmologic research. Breeder pairs were obtained from Charles River (Sulzfeld, Germany). Locally bred female DBA/2J mice were kept under specific pathogen-free conditions at room temperature in a 24-h light/dark cycle. For the DBA/2J strain, a special fat chow containing 5.5% fat instead of 4.5% was provided *ad libitum*. Age-matched control retinas were collected from C57BL/6J mice strain which did not display any ocular abnormalities. The mouse eyes were examined using a microscope (Zeiss OPMI CS).

2.5. Quantification of the signal intensities

For the quantification, images of the stained sections were taken using a Zeiss Axioplan 2 microscope (Zeiss, Jena, Germany) at equal intensity of illumination. The signal strength was analyzed with the openlab software (Improvision, Germany).

2.6. Quantitative RT-PCR

For quantitative RT-PCR analysis, cDNA was generated from 1 μg RNA using Advantage® RT-for-PCR Kit, (Clontech, Mountain View, CA). The PCR was performed in a Light Cycler (Roche, Mannheim, Germany) using Light Cycler Fast Start DNA Master SYBR Green I (Roche, Mannheim, Germany). Primers were: RGMa primers: (5′-TGAACGTGCAAGTCACCAAT-3′)/(5′-CCCGACACCTTCTCTG TGAT-3′) (217 bp), RGMb primers: (5′-GGTCCAGTGCAACTGC TACA-3′)/(5′-CTCTACGTAGCGGCCACTCT-3′) (197 bp), neogenin primers: (5′-CCTCCTATGCCAGTGGTTGT-3′)/(5′-AGGCTTGGAG TCATGTCCAG-3′) (189 bp). Each probe was quantified and normalized to GAPDH using a commercially available primer set and recombinant standard DNA (Search LC, Heidelberg, Germany). The qRT-PCR was performed and evaluated as described previously (Alexander et al., 2006).

Acknowledgements

The authors thank Prof. Dr. Wilhelm K. Aicher (Center for Orthopaedic Surgery, University Hospital Tübingen, Research Laboratory) and Dr. Peter Heiduschka (Section for Experimental Vitreoretinal Surgery, University Eye Hospital of Tübingen) for critically discussing experimental strategies and results.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.modgep.2007.09.002.

References

Alexander, D., Friedrich, B., Abruzzese, T., Gondolph-Zink, B., Wülker, N., Aicher, W.K., 2006. The active form of Leflunomide, HMR1726, facilitates TNF-α and IL-17 induced MMP-1 and MMP-3 expression. Cell Physiol. Biochem. 17, 69–78.

John, S.W., Smith, R.S., Savinova, O.V., Hawes, N.L., Chang, B., Turnbull, D., Davisson, M., Roderick, T.H., Heckenlively, J.R., 1998. Essential iris atrophy, pigment dispersion, and glaucoma in DBA/2J mice. Invest. Ophthalmol. Vis. Sci. 39, 951–962.

Knoll, B., Drescher, U., 2002. Ephrin-As as receptors in topographic projections. Trends Neurosci. 25, 145–149.

Libby, R.T., Anderson, M.G., Pang, I.H., Robinson, Z.H., Savinova, O.V., Cosma, I.M., Snow, A., Wilson, L.A., Smith, R.S., Clark, A.F., John, S.W., 2005. Inherited glaucoma in DBA/2J mice: pertinent disease features for studying the neurodegeneration. Vis. Neurosci. 22, 637–648.

Matsunaga, E., Tauszig-Delamasure, S., Monnier, P.P., Mueller, B.K., Strittmatter, S.M., Mehlen, P., Chedotal, A., 2004. RGM and its receptor neogenin regulate neuronal survival. Nat. Cell Biol. 6, 749–755.

Monnier, P.P., Sierra, A., Macchi, P., Deitinghoff, L., Andersen, J.S., Mann, M., Flad, M., Hornberger, M.R., Stahl, B., Bonhoeffer, F., Mueller, B.K., 2002. RGM is a repulsive guidance molecule for retinal axons. Nature 419, 392–395.

Niederkofler, V., Salie, R., Sigrist, M., Arber, S., 2004. Repulsive guidance molecule (RGM) gene function is required for neural tube closure but not retinal topography in the mouse visual system. J. Neurosi. 24, 808–818.

Oldekamp, J., Kramer, N., Alvarez-Bolado, G., Skutella, T., 2004. Expression pattern of the repulsive guidance molecules RGM A, B and C during mouse development. Gene Expr. Patterns 4, 283–288.

Pasterkamp, R.J., Verhaagen, J., 2001. Emerging roles for semaphorins in neural regeneration. Brain Res. Brain Res. Rev. 35, 36–54.

Rajagopalan, S., Deitinghoff, L., Davis, D., Conrad, S., Skutella, T., Chedotal, A., Mueller, B.K., Strittmatter, S.M., 2004. Neogenin mediates the action of repulsive guidance molecule. Nat. Cell Biol. 6, 756–762.

Schlamp, C.L., Li, Y., Dietz, J.A., Janssen, K.T., Nickells, R.W., 2006. Progressive ganglion cell loss and optic nerve degeneration in DBA/2J mice is variable and asymmetric. BMC Neurosci. 7, 66.

Schmidtmer, J., Engelkamp, D., 2004. Isolation and expression pattern of three mouse homologues of chick Rgm. Gene Expr. Patterns 4, 105–110.

Schwab, J.M., Conrad, S., Monnier, P.P., Julien, S., Mueller, B.K., Schluesener, H.J., 2005. Spinal cord injury-induced lesional expression of the repulsive guidance molecule (RGM). Eur. J. Neurosci. 21, 1569–1576.

Simon, D.K., O'Leary, D.D., 1992. Development of topographic order in the mammalian retinocollicular projection. J. Neurosci. 12, 1212–1232.

Wizenmann, A., Thies, E., Klostermann, S., Bonhoeffer, F., Bahr, M., 1993. Appearance of target-specific guidance information for regenerating axons after CNS lesions. Neuron 11, 975–983.